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Out of the Mouth of Minnows

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The skeleton articulates at specialized junctions, or joints. Although many factors that specify joints are known, how these different mechanisms are integrated to define the joint remains unclear. In this issue of *Developmental Cell*, Askary et al. (2015) utilize zebrafish to identify genetic regulatory mechanisms of joint specification and differentiation.

The establishment of specialized junctures between skeletal elements is key for the dynamic physical properties of the skeleton. These joints vary in structure and function in association with their material composition and the movements they enable. How joints are specified, formed, and maintained are key questions that must be addressed in order to understand skeletal function, as well as the causes of joint dysfunction.

Much is known about the mechanisms underlying initiation and specification of joints. Previous research has focused on specialized joints in the limb, the synovial joints, as they exhibit key innovations that allow for integration of physical force for extended periods of time. Studies of joint development in tetrapod limbs have defined many aspects of how these specialized structures are specified and have allowed for an elegant analysis of morphogenesis and cellular contributions in joints (Decker et al., 2014). While genetic and cellular mechanisms are being continually discovered, many questions remain, such as how cells in the fated inter-joint "zone" differentiate and how joints are shaped. In this issue of *Developmental Cell*, Askary et al. (2015) describe genetic analysis of joint formation and elucidate a regulatory network governing cellular differentiation of joint progenitors. They show that Iroquois homeobox transcription factors (Irx) control the differentiation of joints, specifically through direct regulation of Sox-mediated transcriptional activation of cartilage matrix genes. Previous studies have shown that *Irx1* and *Irx2* genes are expressed in regions abutting cartilaginous domains (including the presumptive joint) during limb development of the chick and mouse (Díaz-Hernández et al., 2013; Zülch et al., 2001), though evidence of their function was lacking. Askary and colleagues (2015) capitalize on the experimental accessibility of the zebrafish to identify a functional role for Irx in joint formation, and they define the foundations of a gene regulatory network specifying differentiation of joint progenitors.

Like all vertebrates, fishes have an articulated skeleton with specialized joints adjoining adjacent elements. However, the joints in teleost fins are not similar to the joints in tetrapod limbs. Rather, the majority of the fin skeleton is made of segmented dermal rays, or lepidotrichia (Figure 1A). These make hinge-like connections between intra-membranous bones and are thus quite different from articulating joints of tetrapod limbs. The cartilaginous appendicular skeleton of the fin is limited to a single series of proximal radials followed by small distal radials. There is little evidence for formation of a joint interface except between the endoskeletal radials and the shoulder (scapulocoracoid, Figure 1B). Interestingly, segmentation of cartilage elements in zebrafish unpaired fins utilizes molecular mechanisms similar to tetrapod limb joints such as *gdf5*, *wnt9*, and *bmp2* (Bruneau et al., 1997; Crotwell and Mabee, 2007). The junctions between these segmented cartilages in fishes, however, do not refine into a specialized structure with stratified cartilage tissue and interdigitating elements. Overall, the lack of directly comparable joint structures in the fins of fishes to those highlighted in tetrapods has limited the use of zebrafish to study joint development. However, two joints in the jaws of fishes resemble the specialized anatomy of the articulating joints in the limb.

The jaws of fishes undergo rapid movement and integration of forces during respiration and feeding, and the structure of these joints is thought to mediate these functions. One joint separates Meckel's cartilage and the palatoquadrate of the jaw, and the other forms between the interhyal, hyoid, and ceratohyal of the branchial skeleton. As large-scale mutagenesis screens have uncovered key genes necessary for formation of the pharyngeal skeleton of the larval zebrafish, including formation of the "jaw" and hyoid joints (Haffter et al., 1996), these mutants set the stage for analyses of the formation of these skeletal structures (e.g., Knight et al., 2004; Miller et al., 2003). Detailed analysis of joint specification and differentiation of their component cellular lineages, however, remains limited.

Using expression screens to identify genes differentially expressed in forming joints of the zebrafish larvae, Askary et al. (2015) identified *irx5a* and *irx7* as co-labeling presumptive positions of the developing hyoid joint. Irx genes were previously known to be associated with chondrogenic differentiation (Díaz-Hernández et al., 2013; Zülch et al., 2001), but their specificity within the hyoid joint led the authors to further investigate their roles. Expression analysis of these genes showed that *irx5a* and *irx7* were responsive to upstream cues for joint formation, similar to those affecting a previously defined regulator of the jaw joint, *nkx3.2/bapx1* (Miller et al., 2003; Nichols et al., 2013). Capitalizing on the ability to alter gene function in the zebrafish, the authors created loss-of-function alleles of both genes. When in combination, *irx5a*::*irx7*

double mutants show specific loss of the hyoid joint with increased staining of chondrogenic matrix. Further analysis using a mammalian micromass culture system demonstrated that Irx7 overexpression repressed cartilage matrix production (type II collagen, aggrecan), whereas Sox9 expression remained unaffected. Importantly, the authors revealed the nature of this repression by showing that both Sox9a and Irx5a directly bind to an enhancer sequence in vitro. Through these analyses, the authors delineate important mechanistic insights into how genetic regulation cascades can mediate not only specification of joints but also differentiation of interzone cartilage cells via differential transactivation of cartilage-specific differentiation factors.

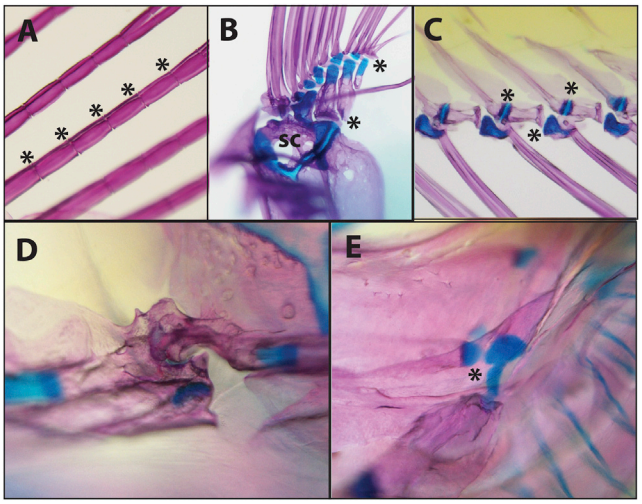

Figure 1. Examples of Joints in the Adult Zebrafish
Skeletons stained with Alcian blue (cartilage) and alizarin red (bone). (A) Joints in the dermal rays of the fins. (B) Proximal and distal radials in the pectoral fin. (C) Segmentation of unpaired fin radials in the anal fin. (D) Hinge of the jaw joint. (E) Hyoid joint. Asterisk denotes position of joint. Sc, scapula. Anterior to the left in all panels.

But are these results informative of the processes of joint development outside of fishes? The specific role of *irx7* beyond this particular joint in the jaw of a fish is not translatable, as this paralogue is unique to fishes. However, all vertebrates share in common an articulated skeleton, and while elaboration and loss of specific developmental-genetic mechanisms are prevalent in particular vertebrate lineages, core processes are conserved. Importantly, Askary et al. (2015) show that the mammalian IRX1 paralog is expressed during the formation of joints in the mouse and is sufficient to suppress chondrogenic markers in cells, similar to the function of zebrafish *irx7*. So, even though the exact players have changed, the mechanisms are likely to be conserved in diverse joints across vertebrates.

Askary et al. (2015) thus uncover central, conserved aspects of the regulation of joint development from an analysis of the zebrafish pharyngeal skeleton. Irx genes may play only a very specific role in the determination of interzone fate. However, these findings expose a direct link of regulatory factors in the establishment of the joint, building components of a genetic regulatory network for joint specification. Importantly, these findings point to the utility of the zebrafish to dissect evolutionarily conserved genetic and cellular mechanisms that regulate joint formation. With the ease of genome-editing technologies, the zebrafish can serve as a model in which we can identify the function of genes implicated in joint development and disease and ask detailed questions regarding the differentiation and diversification of joints and joint tissues. Future work to define the integration of ligaments and muscles with the joint will be important to expand our knowledge of how joints are formed and how they function.

REFERENCES

Askary, A., Mork, L., Paul, S., He, X., Izuhara, A.K., Gopalakrishnan, S., Ichida, J.K., McMahon, A.P., Dabizljevic, S., Dale, R., et al. (2015). Dev. Cell *35*, this issue, 358–365.

Bruneau, S., Mourrain, P., and Rosa, F.M. (1997). Mech. Dev. *65*, 163–173.

Crotwell, P.L., and Mabee, P.M. (2007). Dev. Dyn. *236*, 3111–3128.

Decker, R.S., Koyama, E., and Pacifici, M. (2014). Matrix Biol. *39*, 5–10.

Díaz-Hernández, M.E., Bustamante, M., Galván-Hernández, C.I., and Chimal-Monroy, J. (2013). PLoS ONE *8*, e58549.

Haffter, P., Granato, M., Brand, M., Mullins, M.C., Hammerschmidt, M., Kane, D.A., Odenthal, J., van Eeden, F.J., Jiang, Y.J., Heisenberg, C.P., et al. (1996). Development *123*, 1–36.

Knight, R.D., Javidan, Y., Nelson, S., Zhang, T., and Schilling, T. (2004). Dev. Dyn. *229*, 87–98.

Miller, C.T., Yelon, D., Stainier, D.Y., and Kimmel, C.B. (2003). Development *130*, 1353–1365.

Nichols, J.T., Pan, L., Moens, C.B., and Kimmel, C.B. (2013). Development *140*, 2765–2775.

Zülch, A., Becker, M.B., and Gruss, P. (2001). Mech. Dev. *106*, 159–162.